AMENDMENTS TO THE SPECIFICATION

Please amend the specification and replace paragraph 1 with amended paragraph 1 shown below.

(0001) This application is a ~~divisional~~ <u>continuation</u> application under 37 C.F.R. 1.53(b) and claims priority from U.S. application Serial No. 09/535,675, filed March 23, 2000, now patent No. 6,667,299 B1, which claims priority from abandoned U.S. provisional application Ser. No. 60/190,140, filed March 16, 2000, abandoned U.S. provisional application Ser. No. 60/164,048, filed November 8, 1999, abandoned U.S. application Ser. No. 09/414,905, filed October 8, 1999, abandoned U.S. provisional application Ser. No. 60/140,028, filed June 16, 1999, and abandoned U.S. provisional application Ser. No. 60/126,056, filed March 23, 1999, all of which are incorporated herein by reference.

Replace paragraph 135 at page 40 with the following amended paragraph.

(000135) Another related embodiment is BrEA hemihydrate that is milled to an average particle size of about 0.01-200 μM, or about 0.1-10 μM or about 0.5-5 μM. Average particle size or diameter for milled BrEA hemihydrate may thus be relatively small, e.g., about 0.03-2.0 μM or about 0.1-1.0 μM, or ~~somewhat larger, e.g., about about~~ <u>somewhat larger, e.g., about</u> 0.5-5.0 μM or about 1-5.0 μM. Milled BrEA hemihydrate is suitable for preparing solid formulations and parenteral formulations for human or veterinary use. The milled material facilitates dissolution of BrEA hemihydrate in solvents or excipients and facilitates mixing with solids or solid excipients.

Replace paragraph 202 at page 81 with the following amended paragraph.

(000202) **Groups 37-1 through 37-25-10-6.** These groups comprise each compound named in all of the ~~compound compound groups~~ the compound groups 1 through 36-25-10-6 described above wherein R^1 is not divalent, e.g., is not =O, and it is in the α-configuration, instead of the β-configuration as shown in formula B.

Replace paragraph 206 at page 82 with the following amended paragraph.

(000206) **Groups 41-1 through 41-25-10-6.** These groups comprise each compound named in all of the compound groups 1 through 36-25-10-6 described above ~~wherein R^2 and R^4 is not divalent, e.g., they is not~~ wherein R^2 and R^4 are not divalent, e.g., they are not =O, and they are both in the α-configuration, instead of the β-configuration as shown in formula B.

Replace paragraph 216 at page 90 with the following amended paragraph.

(000216) The formula A compounds, particularly where both R_1 at the 11-position are not hydroxyl, ~~alkoxy or a moiey~~ alkoxy or a moiety that can hydrolyze to a hydroxyl, are generally suitable for use in the methods and compositions that are disclosed herein, e.g., their use to enhance a subject's Th1 immune responses. Methods of administration and dosages are essentially as described herein.

Replace paragraph 238 at page 103 with the following amended paragraph.

(000238) Treatment of 10a with LDA, followed by alkylation of the enolate allows introduction of side chains such as R^{10}, ~~whiich may be, e.g.,~~ which may be, e.g., C1-C20 alkyl (methyl, ethyl), C1-C20 alkenyl (CH_2=CH-$(CH_2)_{0-6}$-), benzyl, -$(CH_2)_{1-4}$-O-$(CH_2)_{0-4}$-CH_3.

Replace paragraph 241 at page 105 with the following amended paragraph.

(000241) Phosphothioesters, R^BO-P(SR^{PR})(O)-O- are generated by treatment of alcohols with the monothio analog of diethylchlorophosphate as described for phosphoesters yielding the phosphothioesters. Carbonates, R^BO-C(O)-O- are generated from the corresponding steroid alcohol using the chloroformate (R^B-C(O)-Cl), e.g., C_{1-20} alkyl, alkenyl or alkynyl chloroformates (e.g. $CH_3(CH_2)_{0-5}$-C(O)Cl). Carbamates, R^B-NH-C(O)-O- are made from steroid alcohols by treatment with isocyanates (R^BN=C=O) or NaOCN in the presence of ~~trifluroroacetic acid. Aminoacid esters~~ trifluroroacetic acid. Amino acid esters, ZNX-CHY-C(O)-O- are generated by coupling the steroid alcohol with the acid chloride of the N-protected amino acid.

Replace paragraph 245 at page 106 with the following amended paragraph.

(000245) Amines and derivatives of amines, e.g., R^BNH-, R^B-C(O)NH-, R^BOC(O)-NH- or R^BO-C(O)-CHR^B-NH- linked to steroid carbon atoms, are typically prepared by standard methods. For example, amines (NH_2-steroid) are generally prepared using the Hoffmann rearrangement (Br_2, NaOH) from the amide (NH_2-C(O)-steroid) or the Curtius rearrangement (NaN_3) from the acid chloride of the steroid. The R^B substituent can subsequently be introduced by alkylation. Steroid alcohols can be used as starting materials under standard Mitsunobu conditions (PPh_3, DEAD) to yield N-Boc sulfonamides using N-(t-butoxycarbonyl)-p-toluenesulfonamide. One can selectively remove either protecting group. Treatment with trifluoroacetic acid affords the sulfonamide (R^B-S(O)(O)-NH-steroid). Alternatively, sodium napthalenide deprotects to give the N-Boc compound. Amines (NH_2-steroid) can be converted to amides (R^BNH-C(O)-steroid) using acyl chlorides (R^B-C(O)-Cl). Treatment with ethyl chloroformate gives the N-carbamate (R^BO-C(O)-NH-steroid). The amine (NH_2-

steroid) can be alkylated with an α-bromoester (R^B-C(O)-CHY-NH_2) to ~~yield the amio acid substituted steroid~~ yield the amino acid substituted steroid (R^B-O-C(O)-CHY-NH-steroid).

Replace paragraph 249 at page 107 with the following amended paragraph.

(000249) Scheme 11. Formula 1 compounds that comprise two or three ring heteroatoms are prepared as shown in the schemes. In the scheme, X is –CH_2-, -NH-, -O-, or -S-; R^{40} is -H or –Br; R^{41} is an organic moiety having about 12 carbon atoms or less, typically C1 – C8 optionally substituted alkyl (e.g., methyl, hydroxymethyl, ethyl, propyl) or C2 – C8 optionally substituted alkenyl having a single double bond (e.g., vinyl) with 1, 2, 3 or ~~more indepenently selected substituents~~ more independently selected substituents (e.g., -OH, -COOH, -O-) and with any substituents that comprise a functional group generally being protected. Preparation of compound 20 from 19 is accomplished using a glycol such as $HOC(CH_3)_2C(CH_3)_2OH$ in acid (H^+) (B.H. Lipshutz et al., *Synth. Commun.* 12: 267, 1982). The use of a bulky protecting group facilitates generation of a double bond at the 5-6 position over the 4-5 position.

Replace paragraph 263 at page 136 with the following amended paragraph.

(000263) More typically hydroxy protecting ~~groups include subtituted~~ groups include substituted methyl ethers, substituted benzyl ethers, silyl ethers, and esters including sulfonic acid esters, still more typically, trialkylsilyl ethers, tosylates and acetates.

Replace paragraph 315 at page 153 with the following amended paragraph.

(000315) Many cancers or malignancies are associated with an unwanted Th2 immune response or a deficient Th1 response. An insufficient Th1 immune response may play a role in the capacity of malignant cells to escape immune surveillance. These conditions include non-small cell lung cancer, bronchogenic carcinoma, renal cell cancer or carcinoma, lymphoma, glioma, melanoma, pancreatic or gastric adenocarcinoma, ~~human pappilomavirus-associated~~ human papillomavirus associated cervical intraepithelial neoplasia, cervical carcinoma, hepatoma and cutaneous T-cell lymphoma (mycosis fungoides, Sezary syndrome).

Replace paragraph 318 at page 155 with the following amended paragraph.

(000318) Insufficient Th1 ~~immune responses are ofter~~ immune responses are often associated with viral infection. Viral infections may arise from DNA or RNA viruses, e.g., herpesviruses, hepadnaviruses, adenoviruses, retroviruses, togaviruses, alphaviruses, arboviruses, flaviviruses, rhinoviruses, papillomaviruses and/or pestiviruses. Exemplary viruses have been described. See, for example B. N. Fields, et al., editors, *Fundamental Virology*, 3rd edition, 1996, Lippencott-Raven Publishers, see chapter 2 at pages 23-57, including table 4 at pages 26-27, table 5 at pages 28-29, chapter 17 at pages 523-539, chapters 26-27 at pages 763-916, chapter 32 at pages 1043-1108 and chapter 35 at pages 1199-1233. As used herein, retroviruses include human and animal viruses, e.g., HIV-1, HIV-2, LAV, human T-cell leukemia virus I ("HTLV I"), HTLV II, HTLV III, SIV, SHIV, FIV, FeLV. Additional viruses, including their genogroups, clades, isolates, strains and so forth, that may establish a virus infection include human hepatitis C virus ("HCV"), human hepatitis B virus ("HBV"), human hepatitis A virus ("HAV"), duck hepatitis virus, woodchuck hepatitis virus, human ("HPV", e.g., HPV 6, HPV 11, HPV 16, HPV 18, HPV 31, HPV 45) or animal papilloma viruses, Poliovirus, Herpes simplex virus 1 ("HSV-1"), Herpes simplex virus 2 ("HSV-2"), human Herpesvirus 6 ("HHV-6"), human Herpesvirus 8 ("HHV-

8"), Dengue virus (types 1-4), Western Equine Encephalitis Virus, Japanese Encephalitis Virus, Yellow Fever Virus and Bovine Viral Diarrhea Virus.

Replace paragraph 340 at page 165 with the following amended paragraph.

(000340) The formula 1 compounds typically interact with one or more biological ~~ligands to efect a biological response~~ <u>ligands to effect a biological response</u>. To facilitate the identification of candidate binding partners for the formula 1 compounds, one can use a radiolabeled formula 1 compound that is linked to a support, usually a solid support, as a means to recover the candidate binding partners. The formula 1 compound can be linked to the support through, e.g., the 3-, 7-, 16- or 17-position of the steroid nucleus. Linking agents are known for such uses and include homobifunctional and heterobifunctional agents, many of which are commercially available. The linker one uses will typically comprise about 2-20 linked atoms. The linked atoms usually comprise mostly carbon, with one, two or three oxygen, sulfur or nitrogen atoms that replace one or more carbon atoms. One can use a cDNA expression library that one has made from suitable cells or tissues as a source of candidate binding partners. The cells or tissues can be obtained from a mammalian or a vertebrate host, e.g., human, mouse, bird, primate, or from other sources, e.g., insects (e.g., Drosophila), other invertebrates (e.g., yeast, bacteria, *Mycoplasma sp., Plasmodium sp.*, *Tetrahymena sp.*, *C. elegans*) or other organism groups or species listed herein or in the cited references. Suitable tissues include skin, liver tissue or cells, including hepatocytes and Kupfer cells, fibrocytes, monocytes, dendritic cells, kidney cells and tissues, brain or other central nervous system cells or tissues, including neurons, astrocytes and glial cells, peripheral nervous system tissues, lung, intestine, placenta, breast, ovary, testes, muscle, including heart or myocyte tissue or cells, white blood cells, including T cells, B cells, bone marrow cells and tissues, lymph tissues or fluids and chondrocytes.

Replace paragraph 601 at page 210 with the following amended paragraph.

(000601) 57C. The method of embodiment 56C wherein the one or more second therapeutic agents is a protease inhibitor, a reverse transcriptase inhibitor, a viral, bacterial or parasite DNA or RNA polymerase inhibitor, an antibacterial antibiotic or an antifungal agent, such as AZT, ddI, ddC, D4T, 3TC, a viral (e.g., HIV) fusion inhibitor, hydroxyurea, nelfinavir, saquinavir, ritonavir, indinavir, chloroquine, a chloroquine analog, amphotericin B, fluconazole, clotrimazole, isoniazid, dapsone, rifampin, cycloserine, erythromycin, a tetracycline antibiotic, vancomycin, ethambutol, pyrazinamide, a flurorquinolone (e.g., ciprofloxacin, norfloxacin), a cephalosporin antibiotic, a β-lactam antibiotic ~~or an aminoglycoside antoibiotic~~ <u>or an aminoglycoside antibiotic</u> (e.g., streptomycin, kanamycin, tobramycin).

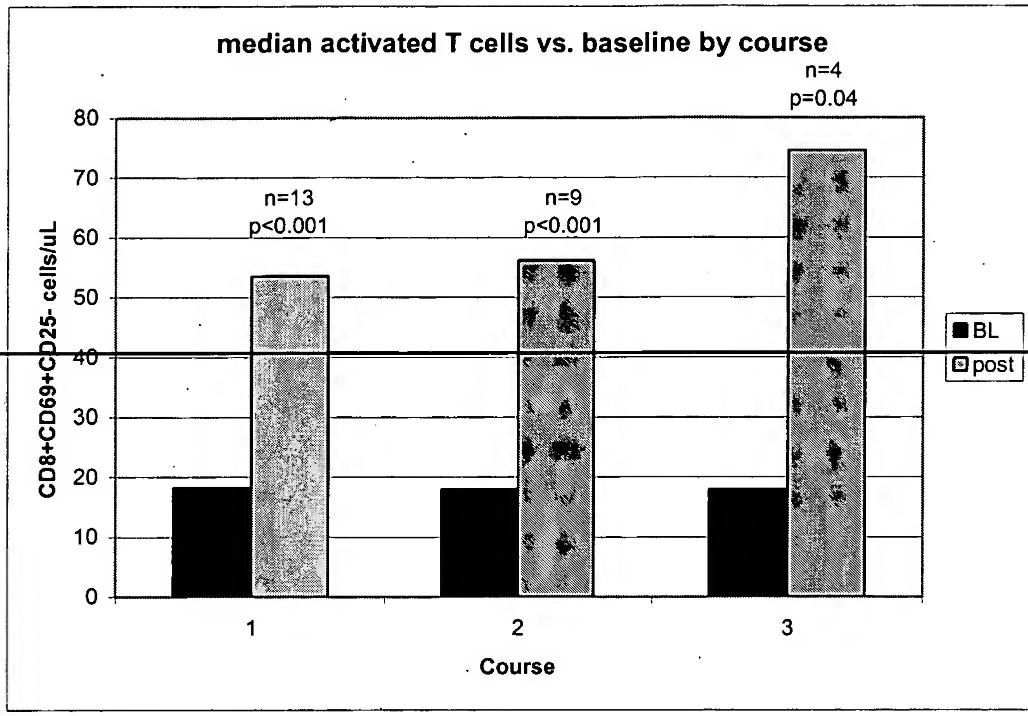
Replace paragraph 695 at page 240 with the following amended paragraph.

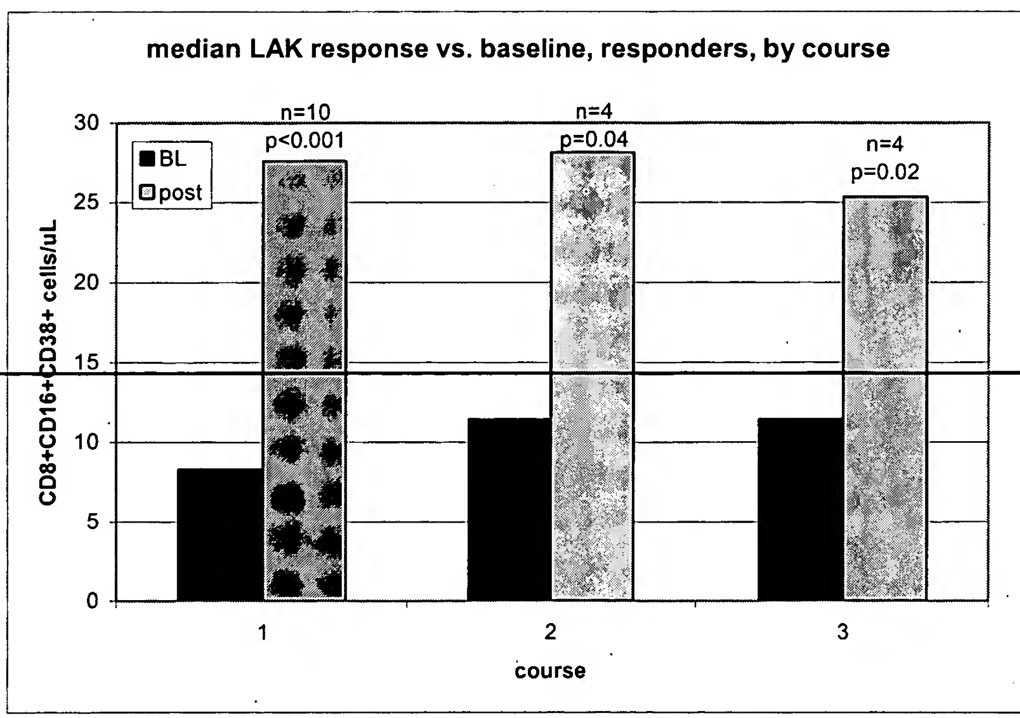
(000695) **In the ~~diagrams~~ tables shown below baseline data is indicated by "BL" or by "pre".**

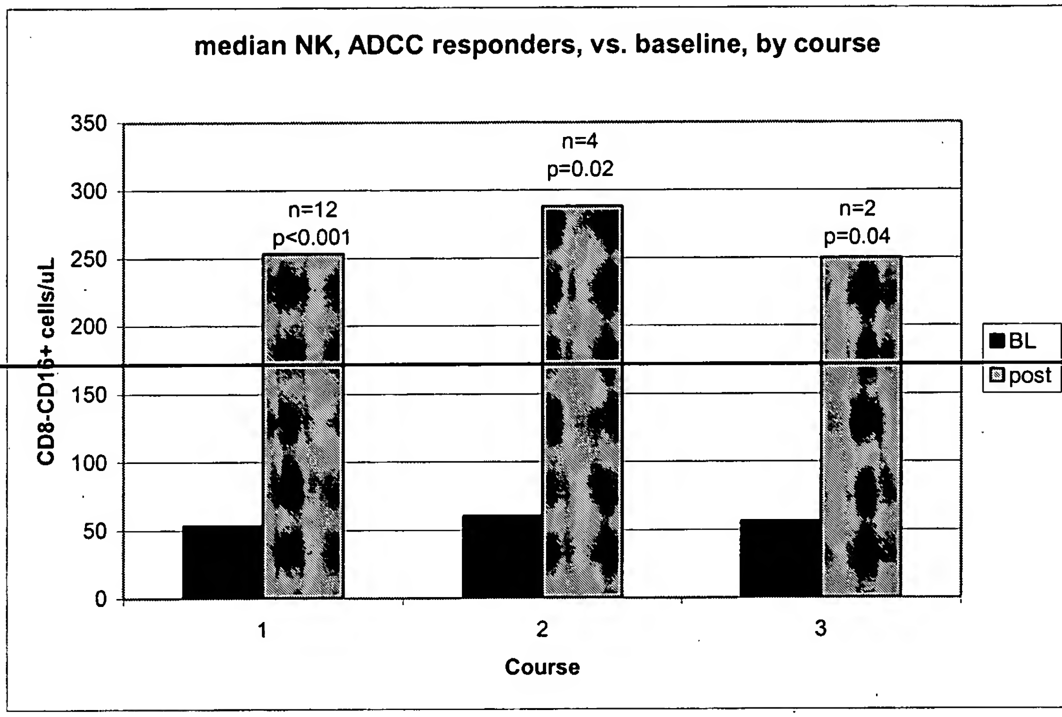
Increased immunophenotypes after BrEA therapy

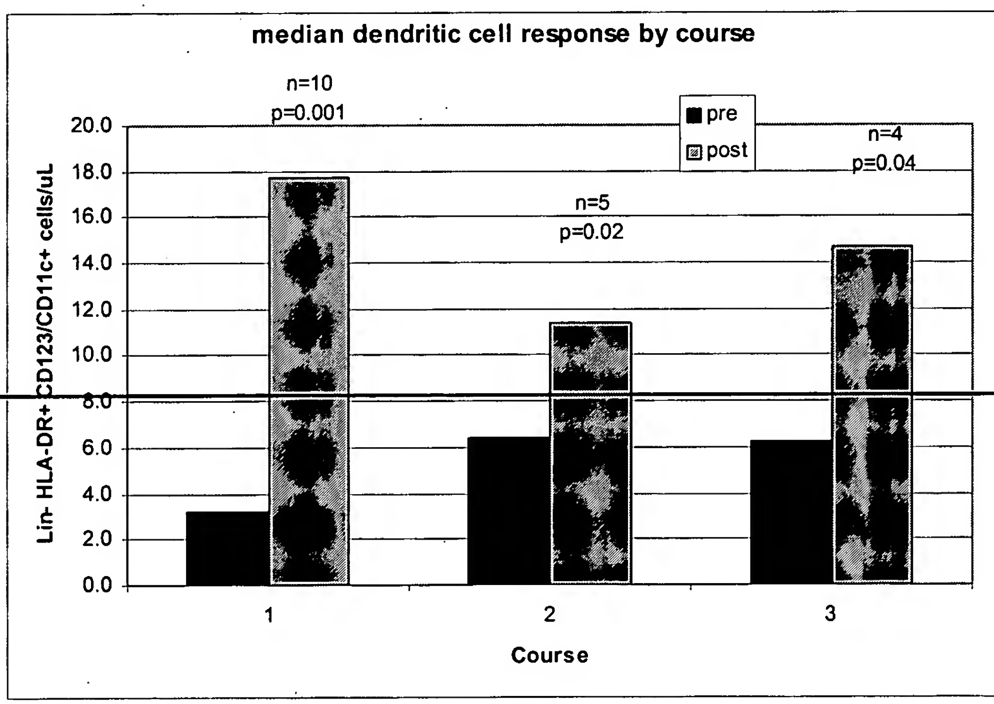
Phenotype	Baseline[a]	Course 1	Course 2	Course 3
CD8+CD69+CD25-	18	54	56	75
n=	(13)	(13)	(9)	(4)
[b]p=		<0.001	<0.001	0.04
CD8+CD16+CD38+	8	27	28	25
n=	(10)	(10)	(4)	(4)
p=		<0.001	0.047	0.02
CD8-CD16+	53	253	288	249
n=	(12)	(12)	(4)	(2)
p=		<0.001	0.02	0.04
Lin- HLA-DR+ CD11c+/CD123+	3.2	17.7	11.4 [c]	14.7[c]
n=	(10)	(10)	(5)	(4)
p=		<0.001	0.02	0.04
IL2+CD4[d]	3.14[e]	29.25	31.42	13.59
n=	13	13	3	4
p=		<0.001	0.09	0.04
IL10+CD4[d]	66	20.9	8.9	15.3
n=	13	13	5	3
p=		0.005	0.005	0.03
Th1 Response[d]	17	66	64	53
n=	(13)	(13)	(5)	(5)
p=		0.001	0.033	0.025

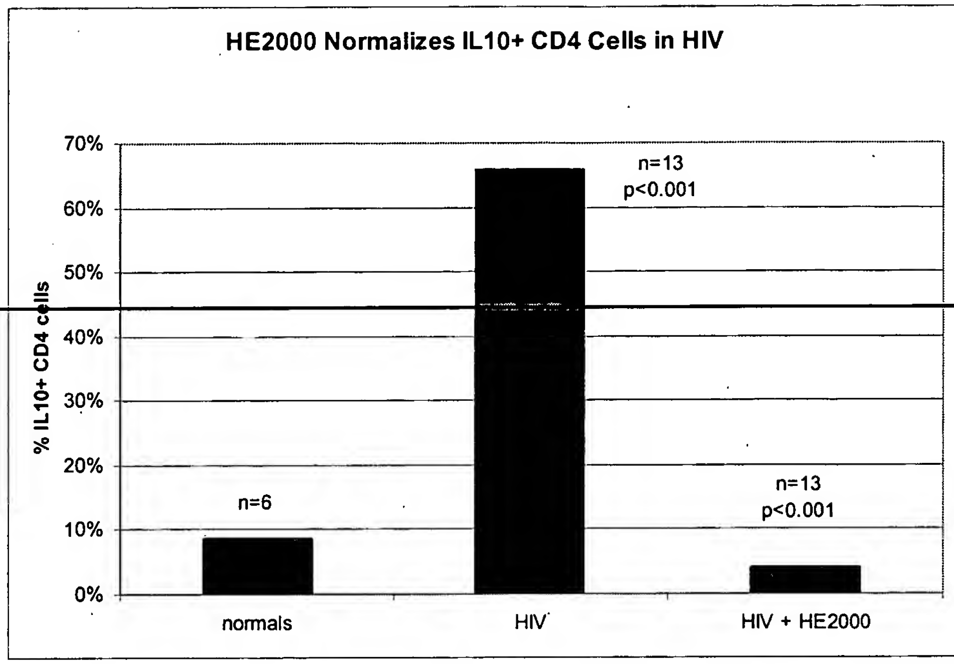
[a]Median values of cells/μL
[b]paired value t test
[c]Test not available at baseline for patients receiving second and third courses, baseline value from initiation of 2nd course = 6.4
[d]% of CD4
[e]Baseline values from day 8 (preceding the first five-day treatment)

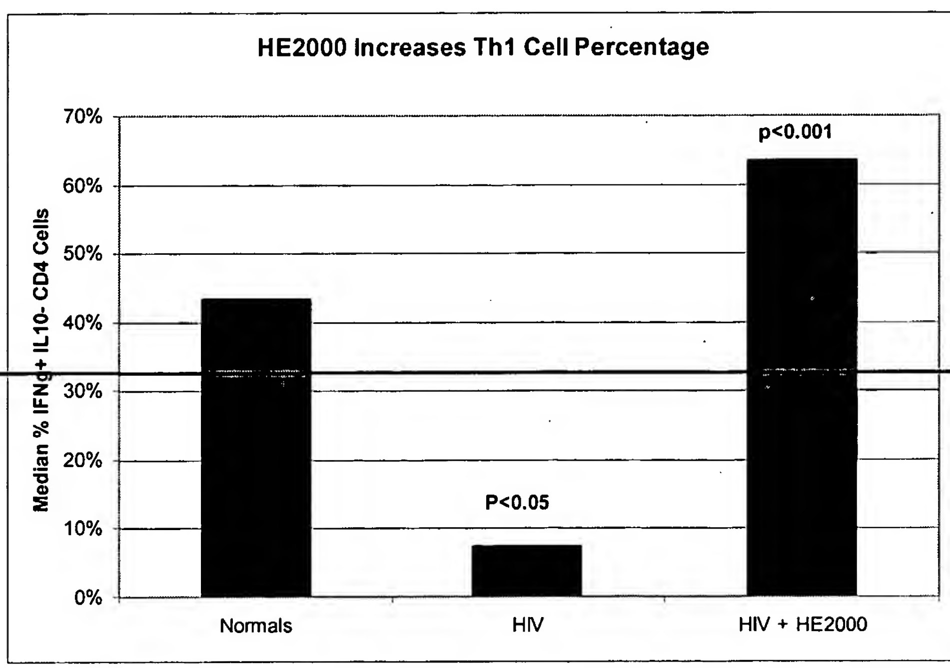

median activated T cells vs. baseline by course
CD8+CD69+CD25- cells/uL
80
70
60
50
40
30
20
10
0
n=13
p<0.001
n=9
p<0.001
n=4
p=0.04
BL
post
1
2
3
Course

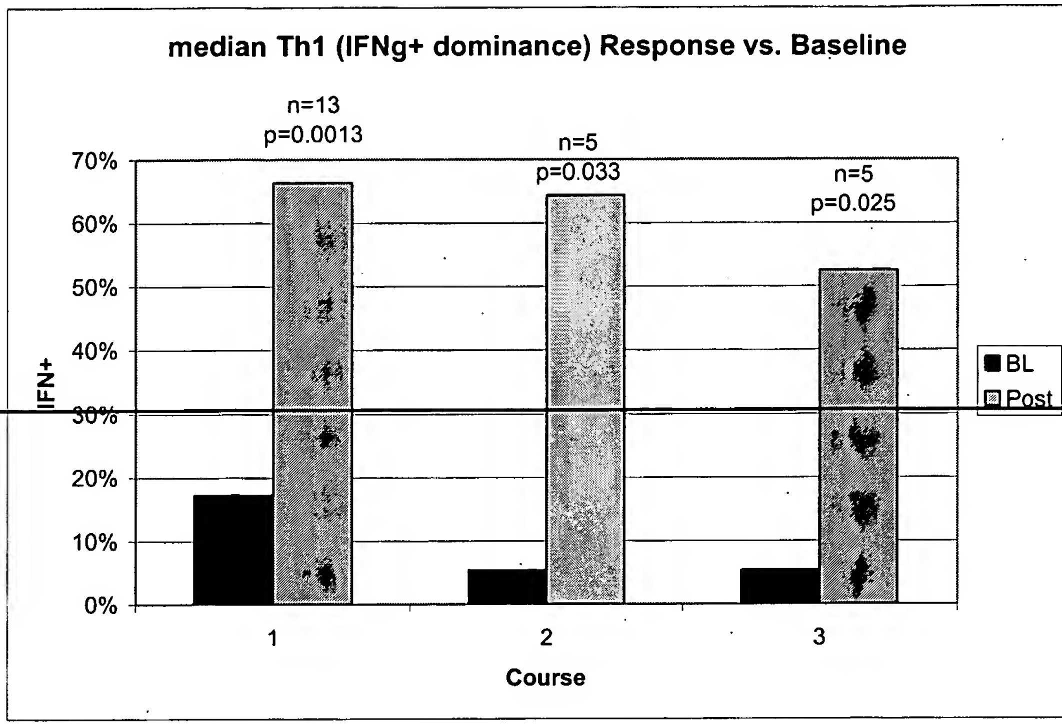

median LAK response vs. baseline, responders, by course
CD8+CD16+CD38+ cells/uL
30
25
20
15
10
5
0
BL
post
n=10
p<0.001
n=4
p=0.04
n=4
p=0.02
1
2
3
course


median NK, ADCC responders, vs. baseline, by course
n=12
p<0.001
n=4
p=0.02
n=2
p=0.04
CD8-CD16+ cells/uL
350
300
250
200
150
100
50
0
1
2
3
Course
BL
post


median dendritic cell response by course
n=10
p=0.001
n=5
p=0.02
n=4
p=0.04
pre
post
Lin- HLA-DR+ CD123/CD11c+ cells/uL
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
1
2
3
Course


HE2000 Normalizes IL10+ CD4 Cells in HIV
70%
60%
50%
40%
30%
20%
10%
0%
% IL10+ CD4 cells
n=6
n=13
p<0.001
n=13
p<0.001
normals
HIV
HIV + HE2000


HE2000 Increases Th1 Cell Percentage
70%
60%
50%
40%
30%
20%
10%
0%
Median % IFNg+ IL10- CD4 Cells
P<0.05
p<0.001
Normals
HIV
HIV + HE2000



Median activated T cells ($CD8^+CD69^+CD25^-$) vs. baseline by course

Course 1 baseline	19 cells/μL	
Course 1	53 cells/μL	$n = 13, p < 0.001$
Course 2 baseline	19 cells/μL	
Course 2	54 cells/μL	$n = 9, p < 0.001$
Course 3 baseline	19 cells/μL	
Course 3	74 cells/μL	$n = 4, p = 0.04$

Median LAK response ($CD8^+CD16^+CD38^+$) vs. baseline, responders, by course

Course 1 baseline	8 cells/μL	
Course 1	26 cells/μL	$n = 10, p < 0.001$
Course 2 baseline	12 cells/μL	
Course 2	27 cells/μL	$n = 4, p = 0.04$
Course 3 baseline	12 cells/μL	
Course 3	25 cells/μL	$n = 4, p = 0.02$

Median NK, ADCC ($CD8^-CD16^+$) responders vs. baseline by course

Course 1 baseline	52 cells/μL	
Course 1	255 cells/μL	n = 12, p < 0.001
Course 2 baseline	59 cells/μL	
Course 2	291 cells/μL	n = 4, p = 0.02
Course 3 baseline	56 cells/μL	
Course 3	249 cells/μL	n = 2, p = 0.04

Median dendritic cell response ($Lin^-HLA\text{-}DR+CD123^+/CD11c^+$) by course

Course 1 baseline	3.2 cells/μL	
Course 1	17.7 cells/μL	n = 10, p = 0.001
Course 2 baseline	6.6 cells/μL	
Course 2	11.6 cells/μL	n = 5, p = 0.02
Course 3 baseline	6.3 cells/μL	
Course 3	14.7 cells/μL	n = 4, p = 0.04

16α-Bromoepiandrosterone normalizes $IL\text{-}10^+$ cells in HIV-infected patients

	% of $CD4^+$ cells that are $IL10^+$	
Normals (HIV^-)	8%	n = 6
HIV^+ patients	64%	n = 13, p < 0.001
treated HIV^+ patients	4%	n = 13, p < 0.001

16α-Bromoepiandrosterone increases Th1 cell proportion in HIV-infected patients

	median % of $CD4^+$ cells that are $IFN\gamma^+$ and $IL10^-$	
Normals (HIV^-)	43%	
HIV^+ patients	8%	p < 0.05
treated HIV^+ patients	63%	p < 0.001

Median Th1 ($IFN\gamma^+$ dominance) response vs. baseline

	median % of $IFN\gamma^+$ T cells	
Course 1 baseline	17%	
Course 1	66%	n = 13, p = 0.00
Course 2 baseline	5%	
Course 2	64%	n = 4, p = 0.0
Course 3 baseline	5%	
Course 3	53%	n = 4, p = 0.0